IDENTIFICATION OF CYP3A4 AS THE ENZYME INVOLVED IN THE MONO-N-DEALKYLATION OF DISOPYRAMIDE ENANTIOMERS IN HUMANS

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(Received January 11, 2000; accepted April 19, 2000)

This paper is available online at http://www.dmd.org

ABSTRACT:
To identify which cytochrome P-450 (CYP) isoform(s) are involved in the major pathway of disopyramide (DP) enantiomers metabolism in humans, the in vitro formation of mono-N-desalkyldisopyramide from each DP enantiomer was studied with human liver microsomes and nine recombinant human CYPs. Substrate inhibition showed that SKF 525A and troleandomycin potently suppressed the metabolism of both DP enantiomers with IC_{50} values for R(−)- and S(+) -DP of <7.3 and <18.9 μM, respectively. In contrast, only weak inhibitory effects (i.e., IC_{50} > 100 μM) were observed for five other representative CYP isoform substrates [i.e., phenacetin (CYP1A1/2), sparteine (CYP2D6), tolbutamide (CYP2C9), S-mephenytoin (CYP2C19), and p-nitrophenol (CYP2E1)]. Significant correlations (P < .01, r = 0.91) were found between the activities of 11 different human liver microsomes for mono-N-dealkylation of both DP enantiomers and that of 6β-hydroxylation of testosterone. Conversely, no significant correlations were observed between the catalytic activities for DP enantiomers and those for the O-deethylation of phenacetin, 2-hydroxylation of desipramine, hydroxylation of tolbutamide, and 4'-hydroxylation of S-mephenytoin. Further evidence for involvement of CYP3A4 in the metabolism of DP was suggested by the low level of activity of CYP3A4 in the human liver microsomes that inhibited the mono-N-dealkylation of both DP enantiomers and 6β-hydroxylation of testosterone almost completely (i.e., >90%), whereas it only weakly inhibited (i.e., <15%) CYP1A1/2- or 2C19-mediated reactions. Finally, the recombinant human CYP3A3 and 3A4 showed much greater catalytic activities than seven other isoforms examined (i.e., CYP1A2, 2A6, 2B6, 2C9, 2D6, 2E1, and 3A5) for both DP enantiomers. In conclusion, the metabolism of both DP enantiomers in humans would primarily be catalyzed by CYP3A4, implying that DP may have an interaction potential with other CYP3A substrates and/or inhibitors.

Disopyramide (DP) is a widely used class IA antiarrhythmic agent (Brogden and Todd, 1987) having a rather narrow therapeutic range (i.e., 2–5 μg/ml or 5–14 μM) (Koch-Weser, 1979). Ragosta et al. (1989) reported that two elderly patients being treated with DP developed ventricular tachycardias and prolongation of QTc intervals on the electrocardiogram immediately after erythromycin, a cytochrome P450 substrate. Laupacis et al. (1997) reported a patient who developed life-threatening arrhythmias immediately after another macrolide antibiotic agent, clarithromycin, to the macrolide-induced inhibition of CYP3A-mediated DP metabolism. In support of this possibility it was reported that ritonavir, another potent CYP3A inhibitor, increased the plasma concentrations of DP and caused cardiac/neurological adverse reactions when coadministered with DP (product information of Norvir, 1997). Thus, in light of a potential risk of drug interaction between DP and CYP3A inhibitors, it would be of value to investigate which CYP isoform(s) are involved in MND formation from the parent drug, DP.

Inasmuch as erythromycin and some other macrolide antibiotics are known to be potent inhibitors of the hepatic CYP3A isoforms (Thummel and Wilkinson, 1998), there is a possibility that CYP3A may be involved in metabolism of DP in humans. The major metabolic pathway of DP is mono-N-dealkylation at the side chain to form mono-N-desalkyldisopyramide (MND) (Lima et al., 1984). We previously demonstrated that enzyme(s) involved in this pathway is susceptible to inhibition by erythromycin and other macrolide antibiotics in an in vitro study performed with human liver microsomes (Echizen et al., 1994), implying that the clinical anecdotes for DP toxicity (Ragosta et al., 1989; Paar et al., 1997) might have been due to the macrolide-induced inhibition of CYP3A-mediated DP metabolism. In support of this possibility it was reported that ritonavir, another potent CYP3A inhibitor, increased the plasma concentrations of DP and caused cardiac/neurological adverse reactions when coadministered with DP (product information of Norvir, 1997). Thus, in light of a potential risk of drug interaction between DP and CYP3A inhibitors, it would be of value to investigate which CYP isoform(s) are involved in MND formation from the parent drug, DP.
preparation was determined by the method of Lowry et al. (1951). The samples were fixed in formalin for histological examination, and the tissue combination of nitrous oxide, halothane, and pancuronium bromide. The liver included nicardipine, nitrendipine, morphine, isosorbide dinitrate, furosemide, another patient (HL-7) drank about 60 ml of ethanol per day for 15 years underwent partial hepatectomy for metastatic liver tumor(s) in the Division of were obtained from 11 patients (aged 45–75, six males and five females) who raised against the purified human CYP3A enzyme protein (Kitada et al., 1992) were obtained from the American Type Culture Collection. Rabbit antibody viruses having cDNA inserts of CYP3A3, 3A4, and 3A5 were described earlier (Nakamura et al., 1996). In this context, we decided to determine the human CYP isoform(s) principally responsible for the major metabolic pathway of each DP enantiomer using human liver microsomes and recombinant human CYP isoforms.

Materials and Methods

Chemicals and Reagents. R(-)- and S(+)-DP phosphate and racemic MND base were generous gifts from Rousel Medica Co. (Tokyo, Japan). The purities of the respective DP enantiomers determined by optical density, melting point, and chiral HPLC were >95% (Echizen et al., 1991). Racemic mephenytoin, 4'-hydroxymephenytoin, and N-desmethylmephenytoin (i.e., nirvanol) were donated by Dr. Künfer (University of Berne, Berne, Switzerland). S- and R-mephenytoin were separated from the racemate by using a Chiralcel OJ column (10 mm, 4.6 × 250 mm; Daicel Chemical Co. Ltd., Tokyo, Japan) according to the method of Yasumori et al. (1990). 2-Hydroxydesipramine oxalate was a gift from Ciha-Geigy (Basel, Switzerland). Propriecipiazine used for the internal standard for the 2-hydroxydesipramine assay was donated by Shionogi Pharmaceutical (Osaka, Japan). Hydroxylbutamide was kindly supplied by Hoechst (Frankfurt, Germany). Timolol HCl, testosterone, desipramine HCl, phenacitin, tolbutamide, erythromycin, sparteine, and tolbutamide were obtained from Sigma Chemical Co. (St. Louis, MO). SKF 525A HCl was obtained from Research Biochemicals Inc. (Wayland, MA). 6β-Hydroxytestosterone was obtained from Steraloids Inc. (Wilton, NH). The MND form of Desipramine, and testosterone, 30 min for desipramine and tolbutamide, and 45 min for respective substrates were: 45 min for DP enantiomers, 20 min for phenacetin metabolism. Thus, R(-)- and S(+)-MND are considered to be derived exclusively from R(-)- and S(+)-DP, respectively. The enzyme reaction was initiated by adding 50 μl of NADPH-generating system consisting of 20 mM glucose 6-phosphate, 5 mM NADP, 40 mM MgCl₂, and 10 U/ml of glucose 6-phosphate dehydrogenase into the incubation mixture that was preincubated at 37°C for 1 min. The incubation mixture contained appropriate amounts of microsomes, 0.1 M sodium phosphate buffer (pH 7.4), 0.1 mM EDTA disodium, and the respective substrates. After an incubation of 250 μM of the final reaction mixture at 37°C in a shaking water bath for 30 to 60 min depending on the substrates, the enzyme reaction was terminated by adding 25 μl of 2 N HClO₄ (for DP) or 100 μl of acetonitrile (for other substrates) into the incubation mixture. Incubation times used for the respective substrates were: 45 min for DP enantiomers, 20 min for phenacitin and testosterone, 30 min for desipramine and tolbutamide, and 45 min for S-mephenytoin. All experiments were performed in duplicate or triplicate. Incubation conditions used for the DP enantiomer metabolism with microsomes obtained from genetically engineered HepG2 cells expressing one of the human CYP3A3, 3A4, and 3A5 were essentially similar to those used for the DP metabolism with human liver microsomes. DP enantiomers and racemate (10 μM each) were incubated separately with the microsomes obtained from HepG2 cells (equivalent to 0.5 mg of protein/ml or 12.1 and 40.7 pmol of P450/ml for CYP3A3 and 3A4, respectively). In addition, DP enantiomers were incubated with the microsomes prepared from uninfected HepG2 cells to determine whether they possess a constitutional catalytic activity for the drug.

Inhibition Study. The effects of coinubation of relatively selective inhibitors or substrates of six distinct human CYP isoforms and of a nonselective CYP inhibitor on the microsomal metabolism of each DP enantiomer were studied separately. Representative inhibitors or substrates used were: phenacetin for CYP1A1/2 (Tassaneeyakul et al., 1993), sparteine for CYP2D6 (Gonzalez, 1990), tolbutamide for CYP2C9 (Relling et al., 1990; Srivastava et al., 1991), S-mephenytoin for CYP2C19 (Whorton et al., 1993; Goldstein et al., 1994), p-nitrophenoil for CYP2E1 (Koop, 1992; Patton et al., 1992), and tolbutamide for CYP3A (Thummel and Wilkinson, 1998). A nonselective CYP inhibitor used was SKF 525A (Schenkman et al., 1972). Each DP enantiomer (32 μM) was incubated with and without one of the inhibitor or substrates at concentrations of 0.1, 1, 10, and 100 μM under the incubation conditions described earlier. The MND formation rates determined in the presence of the respective concentrations of inhibitors or substrates were compared with the control values determined with the incubation of DP.
enantiomers alone and expressed as the percentage of the corresponding control values.

For assessing the inhibitory potency of each CYP isoform-selective substrate or nonselective CYP inhibitor, their concentrations associated with 50% inhibition of the metabolism of the respective DP enantiomers as compared with the corresponding control values (i.e., IC_{50}) were determined based on the concentration-inhibition curves. No substrates or inhibitor were preincubated with NADPH-generating system before initiating the DP metabolism. Experiments were performed with three to four different microsomal preparations that were obtained from distinct human liver samples.

**Assays.** MND formed in the incubation mixture was assayed according to the HPLC-UV detection method reported elsewhere (Echizen et al., 1993) with minor modifications. Briefly, to each reaction-terminated incubation mixture, 50 µl of the internal standard solutions (equivalent to 1 µg of timolol) was added, and the resultant mixture was centrifuged at 10,000g for 5 min. The supernatant was passed through a 0.45-mm (pore size) filter membrane (Gelman Science, Tokyo, Japan), and 50 µl of the filtrate was injected into the HPLC system that consisted of a model L-6000 pump (Hitachi Ltd., Tokyo, Japan), a model 655A-20 automatic sample injector (Hitachi), a reversed-phase HPLC system that consisted of a model L-6000 pump (Hitachi Ltd., Tokyo, Japan) set at 200 nm. Column temperature was maintained at 30°C by a water circulator.

Metabolites of other CYP isoform-selective substrates were determined according to the HPLC-UV absorption methods reported elsewhere (Chiba et al., 1993). The 2-hydroxydesipramine assay was performed according to the method of Koyama et al. (1993) except that the metabolite was detected by a UV absorption method. Briefly, internal standards were used as phenobarbital, proproniazide, chlorpropanide, phenobartitil, and nitrazepam for O-deethylphenacetin (i.e., paracetamol), 2-hydroxydesipramine, hydroxytobutamide, 4'-hydroxymephenytoin, and 6'-hydroxytestosterone, respectively. A reversed-phase HPLC column, CAPCELL PAK C_{18} AG 120 (250 × 4.6 mm internal diameter, 5-µm particle size; Eicom, Kyoto, Japan), and a model 8000 UV absorbance detector (Tosoh, Tokyo, Japan) set at 200 nm. Column temperature was maintained at 30°C by a water circulator.

**ImmunoInhibition Study.** A rabbit antibody raised against the purified human CYP3A was used for the immunoinhibition study. Human liver microsomes (equivalent to 0.025 mg of protein) were preincubated with an incubation buffer containing 0, 1, 2, 3, 4, or 5 µl of the anti-CYP3A serum at 25°C for 30 min. Subsequently, the NADPH-generating system and one of the probe substrates (i.e., 32 µM DP enantiomers, 10 µM phenacetin, 100 µM S-mephenytoin, and 30 µM testosterone) was added to the incubation mixture, and the respective reactions were carried out under the same incubation conditions as described earlier. The microsomal catalytic activities determined with the respective amounts of anti-CYP3A antiserum were compared with those determined with each substrate alone and expressed as the percentage of the corresponding control values.

**Recombinant CYP Study.** cDNA-directed expression of CYP3A3, 3A4, and 3A5 proteins were performed using HepG2 cells and recombinant vaccinia virus according to the methods reported previously (Yamano et al., 1990; Gonzalez et al., 1991). Briefly, HepG2 cells were seeded in tissue flasks and grown to confluence in F-12-supplemented Dulbecco’s modified Eagle’s (DME) and Ham’s nutrient mixture containing 5% (v/v) fetal bovine serum, penicillin, and streptomycin at 37°C in a humidified chamber with 5% CO_2/air. High-titer stock solutions of recombinant vaccinia viruses were diluted to 1 × 10^{10} plaque forming unit (pfu)/ml with PBS and added into the flasks with confluent HepG2 cells. The cells were incubated for 24 h under the conditions described above and harvested by scraping in cold phosphate buffer. Cell pellets were rinsed three times with the buffer and collected by centrifugation at 800g for 5 min at 4°C. The cells resuspended in the phosphate buffer were lysed by sonication on ice. Microsomes were prepared from cell homogenate by differential centrifugation as described earlier. For each batch of the vaccinia-expressed CYP3A proteins, a Soret absorption band being typical of CYPs was confirmed, and its content was measured by the CO binding differential spectrophotometric method (Omura and Sato, 1964). Microsomal protein content was determined by using the BCA protein assay kit (Pierce Chemical Co., Rockford, IL). Western immunoblot analysis was performed for the 20 µg of microsomal protein obtained from each batch of the recombinant HepG2 cells using rabbit polyclonal antibody raised against the purified rat CYP3A2 protein according to the method reported elsewhere (Yamano et al., 1990; Gonzalez et al., 1991). Microsomes containing six other recombinant human CYP isoform proteins (i.e., CYP1A2, 2A6, 2B6, 2C9, 2D6, and 2E1) were obtained from human B lymphoblastoid cells expressing the corresponding CYP isoforms (Gentest Corp., Wobum, MA). The levels of CYP expression for the distinct isoforms were 44.8, 48.6, 66.6, 9.82, 147.0, and 82.4 pmol of P450/mg of protein, respectively.

**Data Analysis.** Data are expressed as mean ± S.D. throughout the text. Correlations between the catalytic activities of human liver microsomes for the selective substrates of six distinct CYP isoforms and those for DP enantiomers were analyzed by the least-squares linear regression method. P < .05 was considered statistically significant.

**Results.**

**Assays.** Under the chromatographic conditions used in this study, no chromatographic peaks that might have interfered with the determination of MND enantiomers and the internal standard (i.e., timolol) were observed in the presence or absence of seven inhibitors or substrates. HPLC assays for the metabolites of the selective substrates of six CYP isoforms and those corresponding internal standards were performed with no possible interfering peaks (chromatograms are not shown). For all metabolites and the internal standards, the mean extraction recoveries from the incubation mixture containing human liver or recombinant microsomes were >95% with coefficients of variation of <6%. Linearity of the microsomal metabolism for the respective CYP substrates and DP enantiomers with regard to the amounts of protein and incubation times have been confirmed in our laboratory, and a part of the data were reported elsewhere (Chiba et al., 1993; Echizen et al., 1994). Results obtained from duplicated incubations did not differ >10% for all the samples. When the incubation was carried out without the NADPH-generation system, no appreciable formation of MND was observed for both DP enantiomers (data not shown).

**Substrate Inhibition Study.** The effects of coinoculation of seven distinct inhibitors or substrates of CYPs on the MND formation from each of the DP enantiomers with human liver microsomes are shown in Fig. 1. The nonselective CYP inhibitor, SKF525A, inhibited the metabolism of both DP enantiomers in a concentration-dependent and enantioselective manner with mean IC_{50} values of 0.4 and 5.4 µM for R(-)- and S(+) DP, respectively. The mean maximum inhibitory effects elicited by SKF525A for the R(-)- and S(+) DP were 89% and 84%, respectively. In addition, a selective inhibitor for CYP3A, troleandomycin, potently inhibited the metabolism of both DP enantiomers in a concentration-dependent and enantioselective manner; the mean IC_{50} values were 7.3 and 15.5 µM and the mean maximum inhibitory effects were 83% and 74%, for R(-)- and S(+) DP, respectively. In contrast, the remaining selective substrates for five CYP isoforms elicited only a weak, if any, inhibitory effect on the DP metabolism. None of them produced inhibitory effects equal to or greater than 50% as compared with the respective control values within the concentration range studied. Phenacetin (10 µM) and sparteine (1 and 10 µM) slightly activated the metabolism of S(+) HUMAN CYPS FOR THE METABOLISM OF DISOPYRAMIDE ENANTIOMERS 939
DP, whereas p-nitrophenol (10 μM) activated the metabolism of R(-)- and S(+)-DP (Fig. 1).

Correlation Study. There were significant (P < .01) correlations between the microsomal catalytic activity for the 6β-hydroxylation of testosterone and that for the mono-N-dealkylation of both DP enantiomers (r = 0.91 for both DP enantiomers) (Fig. 2). The linear regression lines for R(-)- and S(+)-DP were Y = 0.10 · X + 0.0040 and Y = 0.83 · X + 0.014, respectively. The y-intercepts for both DP enantiomers differed insignificantly from 0 (95% confidence intervals for R(-)- and S(+)-DP were -0.028 to 0.036 and -0.004 to 0.032, respectively). In contrast, no significant correlations were observed between the microsomal activities for the four selective substrates of human CYP isoforms and those for both DP enantiomers (Fig. 2).

Immunoinhibition Study. The anti-human CYP3A serum elicited a potent inhibitory effect on the microsomal metabolism of both DP enantiomers in a dose-dependent manner (Fig. 3): the maximum inhibitory effects produced by 5 μl of antiserum were 100 and 95% for R(-)- and S(+)-DP, respectively, as compared with the corresponding control values. In contrast, the maximum volume of the anti-CYP3A serum (i.e., 5 μl) elicited only weak inhibitory effects on the microsomal metabolism of phenacetin (i.e., 9%) and S-mephenytoin (i.e., 15%) as compared with the respective control values.

Recombinant CYP Study. Western immunoblot analysis showed that the microsomes obtained from HepG2 cells genetically engineered for expressing one of the three distinct human CYP3A isoforms exhibited a single polypeptide band with approximate molec-
ular weights being compatible with the CYP3A isoforms (data not shown). Immunoblot analysis for the microsomes prepared from the control HepG2 cells showed no protein band that cross-reacted with anti-rat CYP3A2 antibody, and the microsomes showed no reduced CO binding spectrum (data not shown). With the use of the current vaccinia virus-based expression system, levels of CYP isoforms expressed in HepG2 cells ranged from 10 to 20 pmol/mg of total cell lysate protein. Although the microsomes prepared from the HepG2 cells expressing human CYP3A3 and 3A4 showed a substantial mono-N-dealkylation activity for both DP enantiomers and racemate, CYP3A5 showed much less activity than CYP3A3 and 3A4 (Fig. 4) despite that these three CYPs demonstrated largely comparable catalytic activities for the 6β-hydroxylation of testosterone (i.e., 3.0–7.5 pmol/min/pmol of P450). Interestingly, the recombinant CYP3A3 and 3A4 showed a preferentially greater catalytic activity for \( S^{(+)} \)-over \( R^{(-)} \)-DP. As to six other recombinant CYP isoforms examined, only CYP 2C9 showed a small, albeit measurable, catalytic activity for both DP enantiomers (Fig. 4).

**Discussion**

This is the first attempt for identifying which CYP isoform(s) are responsible for the major metabolic pathway of DP enantiomers (i.e., mono-N-dealkylation) in humans. Using human liver microsomes and anti-CYP3A serum we revealed that the mono-N-dealkylation of both DP enantiomers is mediated primarily by CYP3A (Figs. 1–3). With

**FIG. 2.** Correlations between catalytic activities toward five representative CYP isofrom substrates and those for \( S^{(+)} \)- and \( R^{(-)} \)-DP determined with 11 distinct human liver microsomal preparations.

(□) and (●) represent the catalytic activities for \( R^{(-)} \)- and \( S^{(+)} \)-DP, respectively. Detailed experimental conditions (e.g., substrate concentrations and incubation times) are given under *Materials and Methods*. Note that significant correlations (\( r = 0.91, P < .01 \) for both DP enantiomers) were observed between the catalytic activity toward CYP3A specific substrate (i.e., 6β-hydroxylation for testosterone) and mono-N-dealkylation of both DP enantiomers. NS = not significant.

**FIG. 3.** Immunoinhibition study on the mono-N-dealkylation of the respective DP enantiomers by anti-CYP3A serum performed with human liver microsomes.

Note that substantial and nearly superimposable inhibitions in a concentration-dependent manner were observed not only for the mono-N-dealkylation of \( R^{(-)} \)- and \( S^{(+)} \)-DP (□ and ●, respectively) but also for 6β-hydroxylation of testosterone (▲). In contrast, only few changes were observed for phenacetin O-deethylation (△) and 4β-hydroxylation of S-mephénytoïn (●) mediated by CYP1A2 and CYP2C19, respectively. Data are means of duplicate experiments.
with human liver microsomes exhibited a biphasic enzyme kinetic shown that the mono-phenacetin, diazepam, and imipramine as model compounds. We have reported by Chiba et al. (1994) and Tassaneeyakul et al. (1993) using which are largely compatible not only with its therapeutic plasma concentration of the drug (i.e., 100

but it was mediated primarily by CYP3A4/5 at a suprapharmacologi-
dominated by CYP2C19, being compatible with previous in vivo data,

In support of this contention, there were no significant correlations
between the microsomal activities for the mono-

N-dealkylation of both DP enan-

omers did not differ significantly from 0. Because the microsomal

activities of CYP1A2, CYP2D6, CYP2C9, and

Wilkinson, 1998). In contrast, the representative substrates for the five

other human CYP isoforms (i.e., CYP1A2, CYP2D6, CYP2C9,

CYP2C19, and CYP2E1) elicited little or only a weak inhibitory effect on the metabolism of both DP enantiomers. Taken together, the results obtained from the substrate inhibition study (Fig. 1) suggest that the CYP3A isoform(s) are likely be responsible for the DP metabolism in humans.

There were significant (r = 0.91, P < .01) correlations between the microsomal activities for the mono-N-dealkylation of both DP enantiomers and that for the 6β-hydroxylation of testosterone (Fig. 2). In addition, the y-intercepts of the regression lines for both DP enantiomers did not differ significantly from 0. Because the microsomal activity for the 6β-hydroxylation of testosterone is a result of CYP3A (Waxman et al. 1988), the microsomal metabolism of both DP enantiomers appears primarily to be mediated by this CYP subfamily. In support of this contention, there were no significant correlations between the microsomal activities for the metabolism of DP enantiomers and those for four other distinct metabolic pathways representing the microsomal activities of CYP1A2, CYP2D6, CYP2C9, and CYP2C19, respectively (Fig. 2). Nonetheless, we cannot totally eliminate the possibility that certain CYP(s), which were not assessed in this study, may have a significant contribution to the metabolism of DP enantiomers.

The specific anti-CYP3A serum (Kitada et al., 1992; Nakasa et al., 1993) added to the human liver microsomes inhibited the mono-N-dealkylation of both DP enantiomers as well as 6β-hydroxylation of testosterone (Fig. 3). The immunoinhibition curves for both DP enantiomers and testosterone were almost superimposable among each other and the microsomal activities of both substrates were almost completely abolished at the maximum volume (i.e., 5 μl) of the anti-CYP3A serum. In contrast, only small inhibitory effects (i.e., <15%) were observed on phenacetin O-deethylation and 5-mepheno-
toin 4'-hydroxylation (Fig. 3) by the maximum amount of the anti-CYP3A serum, indicating that the anti-CYP3A serum used in this study was specific for CYP3A and that the metabolism of both DP enantiomers would be mediated almost exclusively by this CYP subfamily. However, we should interpret the results of the immunoinhibi-
tion study with some reservation because it is quite difficult, if not impossible, to exclude the possibility that the anti-CYP3A serum used might inhibit other human CYP(s) other than those examined herein (i.e., CYP1A2 and CYP2C19).

The in vitro study assessing the metabolic activities of the nine distinct recombinant human CYPs toward DP enantiomers demonstrated that CYP3A3 and 3A4 possessed (by far) greater catalytic activities than any other CYP isoforms, and that the metabolism of DP

inhibitor, SKF525A (Schenkman et al., 1972), produced a potent inhibitory effect on the microsomal metabolism of both DP enanti-

omers (Fig. 1). In addition, no appreciable MND formation was observed from both DP enantiomers unless the NADPH-generation system was added to the microsomal incubation mixture. These find-
ings suggest that CYP enzyme(s) are involved in the oxidative mono-N-dealkylation of both DP enantiomers with human liver microsomes. The observation that troleandomycin showed a potent inhibitory effect on the metabolism of both DP enantiomers is compatible with our previous finding that macrolide antibiotics are potent inhibitors for the in vitro mono-N-dealkylation of DP racemate (Echizen et al., 1993) and enantiomers (Echizen et al., 1994) with human liver microsomes. Although macrolide antibiotics are metabolized selectively by CYP3A, some of them (e.g., troleandomycin and erythromycin) were demonstrated to form a stable nitrosoalkane complex with the heme moiety of CYP3A (Periti et al., 1992), thereby potently inhibiting the metabolism of many CYP3A substrates in humans (Thummel and Wilkinson, 1998).
with these two CYP isoforms was enantioselective: $S(+)\text{-DP}$ was metabolized preferentially over $R(-)\text{-DP}$ at therapeutically relevant substrate concentration (i.e., 10 $\mu$M) (Fig. 4). This finding was consonant with that obtained from the previous in vitro study performed with human liver microsomes (Echizen et al., 1994) and in vivo human studies (Lima and Boudoulas, 1985; Echizen et al., 1991). In addition, the finding that both DP enantiomers are catalyzed largely by the same CYP isoforms would explain the reason why the metabolic competition was observed between the DP enantiomers during the in vitro study performed with human liver microsomes (Echizen et al., 1994). The reason that CYP3A5 possessed much lower catalytic activity than CYP3A3 and 3A4 toward both DP enantiomers remains unknown. However, different catalytic properties toward endogenous and exogenous substrates among CYP3A isoforms (i.e., CYP3A4, 3A5, and 3A7) were reported by Ohmori et al. (1998). The CYP3A subfamily is known to be expressed most abundantly (i.e., from 10–60% of total CYPs) in human liver and to play a pivotal role in the oxidative metabolism of many clinically important drugs (Thummel and Wilkinson, 1998). Among the four distinct CYP3A isoforms so far cloned (i.e., CYP3A3, 3A4, 3A5, and 3A7), CYP3A4 would be a major isoform in adult humans. Although CYP3A5 is polymorphically expressed in only approximately 10 to 20% of the adult liver (Aoyama et al., 1989; Wrighton et al., 1990), CYP3A7 is expressed exclusively in the fetal liver (Komori et al., 1990). CYP3A3 appears to constitute a very minor form in human liver (Bork et al., 1989). Collectively, we are tempted to speculate that CYP3A4 would be the major CYP isoform responsible for the hepatic metabolism of DP enantiomers in the majority of adult humans and adult patients given DP as a racemate.

Assuming that CYP3A4 is involved mainly in the hepatic metabolism of DP enantiomers, it can be anticipated that DP might be susceptible to a metabolic inhibition by certain CYP3A-selective inhibitors and/or substrates (e.g., erythromycin and ritonavir). In this context, it is of interest that there are clinical reports that these CYP3A-oriented metabolic inhibitors/substrates may give rise to cardiac and/or neurological adverse reactions (Ragosta et al., 1989; Paar et al., 1997; product information of Norvir, Abbott Laboratories, North Chicago, IL, 1997). Howevet, DP is eliminated via both the hepatic metabolism and renal elimination to a similar extent in healthy young subjects (Lima et al., 1984). Thus, one may assume that the metabolic inhibition can cause up to a 50% reduction in the systemic clearance of the drug. Although this clearance reduction of DP may not lead to the substantial change in plasma DP concentrations in patients with normal renal function, as to patients with impaired renal function due either to renal diseases or aging, the elimination of DP would depend primarily on the hepatic metabolism. Thus, these patients could be considered more susceptible to drug interaction with CYP3A4 inhibitors (e.g., erythromycin, clarithromycin, azole antifungals, and certain HIV-1 protease inhibitors) via a metabolic inhibition than those with normal renal function.

In conclusion, the results of this study suggest that the in vivo hepatic metabolism of DP enantiomers in humans is most likely to be mediated by CYP3A4. Because it is known that CYP3A enzymes are involved in the oxidative metabolism of numerous therapeutically important drugs (Periti et al., 1992; Thummel and Wilkinson, 1998), further studies are required to assess if DP would cause a clinically relevant metabolic interaction with any of CYP3A4 inhibitors particularly in patients with impaired renal function or in geriatric patients. In this context, it also remains to be studied if the in vitro recombinant CYP3A system would be a useful tool in forecasting an in vivo metabolic interaction between DP and other CYP3A substrates or inhibitors.

Acknowledgments. We thank K. Manabe and A. Saito for their technical assistance, Dr. E. Koyama for her useful comments and suggestions in the preparation of the manuscript, and Dr. T. Ohmori for his generous supply of the specific antibody against human CYP3A.


